

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1. **(currently amended):** A plasmid comprising the following functional units:

- a prokaryotic origin of replication,
- a marker sequence,
- two specific recombinase recognition sequences and
- a multiple cloning site, characterised in that it comprises a gene coding for a sequence specific recombinase,

whereby the units are arranged on the plasmid in such a way that the plasmid is divided into a miniplasmid and a minicircle upon expression of the sequence specific recombinase, said miniplasmid comprising the prokaryotic origin of replication, the marker sequence and the gene for the sequence specific recombinase and said minicircle comprising the multiple cloning site,

wherein the sequence specific recombinase is ParA resolvase.

2. **(original):** The plasmid according to claim 1, characterised in that a gene coding for a specific protein, preferably a therapeutically useful protein, is inserted into the multiple cloning site.

3. **(currently amended):** The plasmid according to claim 1 or 2, characterised in that it further comprises a minicircle identification sequence for the identification and isolation

of the minicircle and/or a miniplasmid identification sequence for the identification, isolation and removal of the miniplasmid.

4. **(canceled).**

5. **(currently amended):** The plasmid according to claim 3-~~or~~4, characterised in that

the identification sequence is a sequence which is able to specifically bind to a protein in order to form a stable DNA-protein complex.

6. **(original):** The plasmid according to claim 5, characterised in that the identification sequence is a lac operator site which specifically binds to a LacI repressor protein.

7. **(original):** The plasmid according to claim 5 or 6, characterised in that it further comprises a gene coding for the protein which forms the DNA-protein complex, preferably a gene coding for the LacI repressor protein.

8. **(currently amended):** The plasmid according to ~~any one of~~ claims 5-~~to~~-7, characterised in that the plasmid comprises a sequence coding for a hydrophobic membrane anchoring peptide.

9. **(canceled).**

10. **(currently amended):** The plasmid according to ~~any one of~~ claims 1-to-9, characterised in that the specific recombinase recognition sequences are selected from the group consisting of lambda attachment sites (*att* sites) and resolution sites (*res* sites) from Multimer Resolution Systems.

11. **(currently amended):** The plasmid according to ~~any one of~~ claims 1-to-10, characterised in that it further ~~comprises~~comprising a regulatory element for the expression of the recombinase.

12. **(original):** The plasmid according to claim 11, characterised in that the regulatory element for the recombinase comprises a strong promoter.

13. **(original):** The plasmid according to claim 12, characterised in that the regulatory element is a transcriptional control system of an araB promoter of an araBAD operon.

14. **(currently amended):** The plasmid according to ~~any one of~~ claims 1-to-13, characterised in that the marker gene is an antibiotic resistance gene.

15. **(currently amended):** The plasmid according to ~~any one of~~ claims 1-to-14, characterised in that the prokaryotic origin of replication is a high copy number origin of replication, preferably from plasmid pUC19.

16. **(currently amended):** The plasmid according to ~~any one of~~ claims 1-~~to~~-15, characterised in that ~~it wherein the minicircle~~ comprises an origin of replication ~~on the minicircle.~~

17. **(currently amended):** A kit for the production of a therapeutically useful DNA molecule, characterised in that it ~~comprises comprising~~

- the plasmid according to ~~any one of~~ claims 5-~~to~~-15 and
- a protein which is able to bind to the identification sequence of the plasmid in order to form a stable DNA-protein complex, whereby the protein is optionally immobilised to a solid support.

18. **(original):** The kit according to claim 17, characterised in that the protein is a LacI repressor protein, preferably a mutant LacI repressor protein.

19. **(currently amended):** The kit according to claim 17-~~or~~-18, characterised in that the protein is fused to a tag for the immobilisation to a solid support.

20. **(currently amended):** The kit according to claim 17-~~or~~-18, characterised in that the protein is fused to a hydrophobic, membrane anchoring peptide.

21. **(currently amended):** The kit according to claim 20, characterised in that it ~~comprises~~ wherein the plasmid comprises with an inducible lysis gene and further comprises a culture of recombinant bacteria transfected with said plasmid, respectively.

22. **(original):** The kit according to claim 21, characterised in that the lysis gene is the lysis gene E of bacteriophage PhiX174.

23. **(currently amended):** The kit according to ~~any one of claims 16 to 22~~¹⁷, characterised in that it comprises further comprising a culture of bacteria specific for the expression and function of the recombinase.

24. **(currently amended):** The kit according to ~~any one of claims 16 to 23~~¹⁷, characterised in that it further comprises comprising arabinose.

25. **(currently amended):** A minicircle characterised in that it is derivable^{derived} from the plasmid according to ~~any one of claims 8 to 16~~¹⁷, and in that it comprises^{comprising}

- a multiple cloning site and
- a gene coding for a therapeutically useful protein inserted into the multiple cloning site,

whereby the minicircle is attached to a bacterial ghost over a hydrophobic membrane anchoring peptide.

26. **(original):** A pharmaceutical composition comprising the minicircle according to claim 25 and a pharmaceutically acceptable carrier.

27. (currently amended): A method for the production of a therapeutically useful DNA molecule, ~~characterised in that it comprises~~comprising the following steps

- transfecting the plasmid according to ~~any one of~~ claims 2 to 15 into bacteria which are able to replicate the plasmid,
- culturing the bacteria during which the recombinase is expressed so that miniplasmids and minicircles are produced and
- isolating the minicircles.

28. (currently amended): The method according to claim 27, ~~characterised in that~~wherein the minicircles are isolated by using ~~the~~a minicircle identification sequence.

29. (currently amended): The method according to claim 27 or 28, ~~characterised in that~~wherein the minicircles are isolated by immobilisation to a solid support, ~~preferably a chromatography column.~~

30. (currently amended): The method according to ~~any one of~~ claims 27 to 29, characterised in that the recombinase is expressed upon induction of the regulatory element, preferably by adding arabinose to the culture medium.

31. (currently amended): The method according to ~~any one of~~ claims 27 to 28, ~~characterised in that~~wherein a protein recognising the minicircle identification sequence is also

further expressed in the bacteria and anchored in the bacterial membrane so as to be capable of binding to the minicircles.

32. (new): The method according to claim 29, wherein the minicircles are isolated by a chromatography column.